Let's PLAy: Bioproduction of Poly-Lactic Acid

iGEM EVRY 2016

The plastic problem

Capture on huge amount of plastic

Some plastic facts

- **280 million tons**: average **amount** of plastic produced each **year**, worldwide.
- **1 000 years**: the typical **life-span** of a plastic bottle!
- Up to **450 years** : time of **degradation** of plastic bags in the environment.

Bioplastics: an alternative?

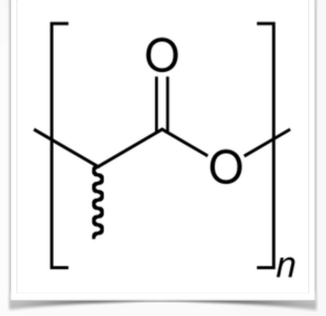
- Bioplastics are **biodegradable** polymers.
- Compared to conventional plastics based on petroleum, they are derived from **renewable biomass sources.**

Bioplastic symbol:

Our solution: PLA bioproduction in *P.putida*

PLA: Poly-lactic acid

- Biodegradable polymer and thermoplastic.
- Used for food packaging and for biomedical applications.



Poly-lactic acid (PLA) structure

How? Using Pseudomonas putida as chassis

Before, Jung *et al.*, 2010 used *E.coli*[1]

	E. coli	*B. subtilis*	*P. putida* KT2440
Ease of manipulation	***	***	***
Safety	***	***	***
Lactate production	*	***	**
Polymer formation	*	*	***

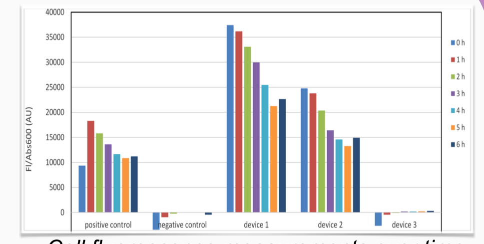
Bioproduction is more eco-friendly and economical than current chemical synthesis.

InterLab

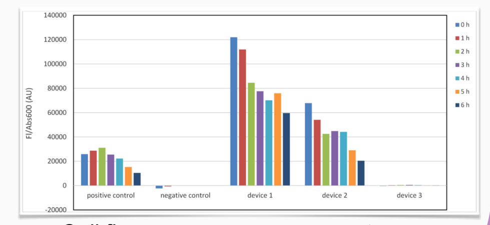
We participated in the InterLab, studying **GFP expression** for 3 devices in pSB1C3:

- J23101.B0034.E0040.B0015
- J23106.B0034.E0040.B0015
- J23117.B0034.E0040.B0015

Control +: I20270
Control -: R0040



Cell fluorescence measurements over time



Cell fluorescence measurements on TB media+betain/sorbitol.

Beyond the lab

In Human Practices, we:

- Investigated **public awareness** on **bioplastics** & **legislative restrictions** on plastics.
- Presented our **MOOC** to high school students.
- Experimented PLA degradation as a **skepticism** exercise.

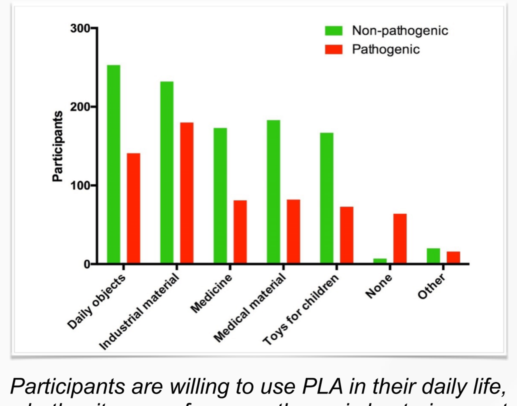
Banning plastics:

Focusing mainly on European & French legislations, we wondered **how banning plastics** would affect the **market of bioplastics**. We concluded that banning petroleum-based plastic may lead to a **decrease** of bioplastic prices.

Educational Survey:

Is our public ready to use PLA?

Yes. If they had to choose between PLA and another petroleum-based product, **88.3%** of our respondents would **choose PLA.**



Participants are willing to use PLA in their daily life, whether it comes from a pathogenic bacteria or not.

Investigation on our project showed that development of more **efficient production** processes would lead to **costs reductions** through economies of scales. We integrated it on our **DIY Bioprocess.**

Public Engagement:

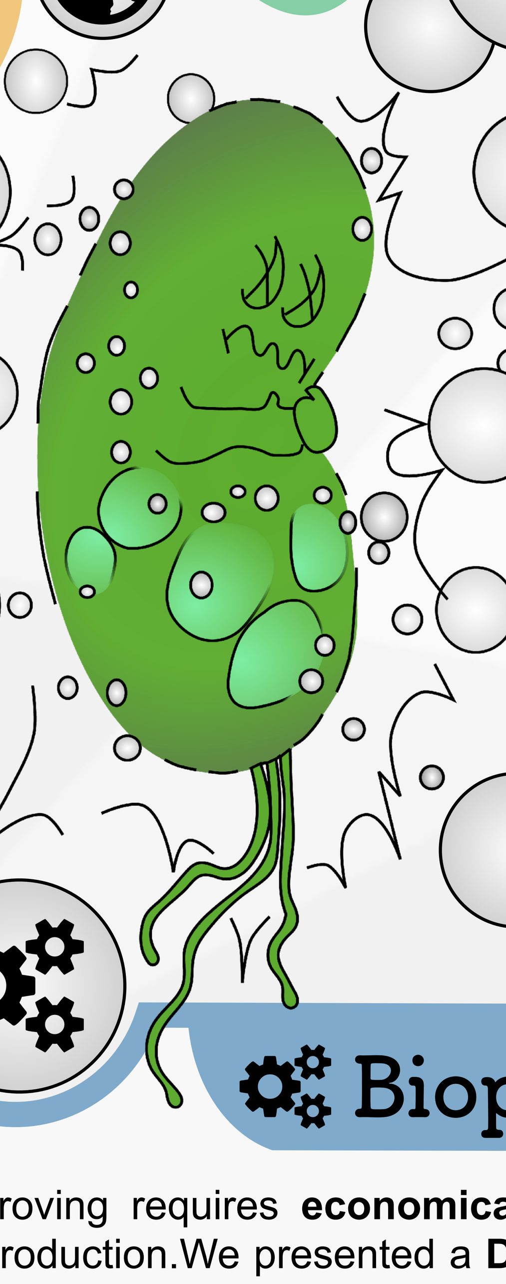
- High school presentations
- Bioplastics Survey and MOOC
- Crowdfunding campaign
- Improvement of iGEM Wikipedia pages

MOOC:

Together with iGEM IONIS team, we organized **The European Experience**, an **European iGEM Meet-Up** allowing European teams to meet during one weekend in **Paris** for sharing ideas and learning about synthetic biology.

The European Experience

Participants on the European Experience.



Design

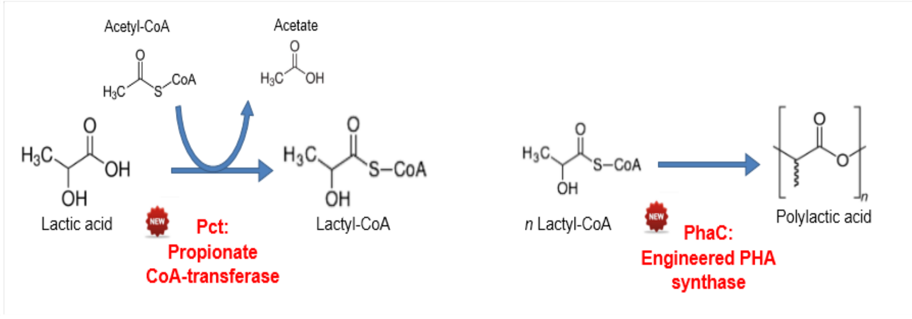
To increase PLA yield, an **evolved lactate dehydrogenase** (D-LDH*)[2] allowed increasing quantity of precursor: lactate.

Basic Pathway:

To bioproduce PLA in *P.putida* we needed two enzymes[1]:

- **Pct**, converting lactate into lactyl-coA.
- **PhaC***, to polymerize monomers of lactyl-CoA into PLA.

* Engineered.

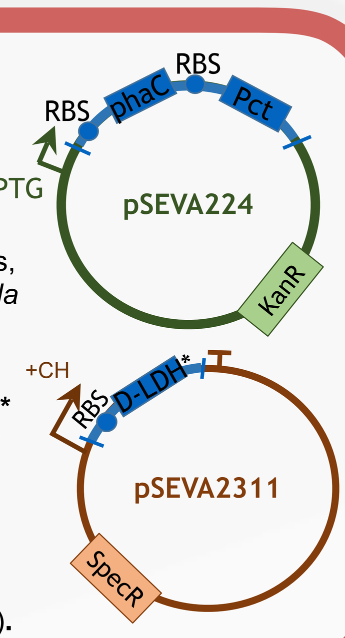


Enzymes allowing PLA synthesis: Pct and PhaC*

Plasmid constructs:

pSEVA are standardized plasmids, working well in *P.putida* [3].

- pSEVA 224: Expression of **PhaC*** and **Pct**, induced by **IPTG**.
- pSEVA 2311: Expression of **D-LDH***, induced by cyclohexanone (**CH**).



Parts & Collabs.

Parts:

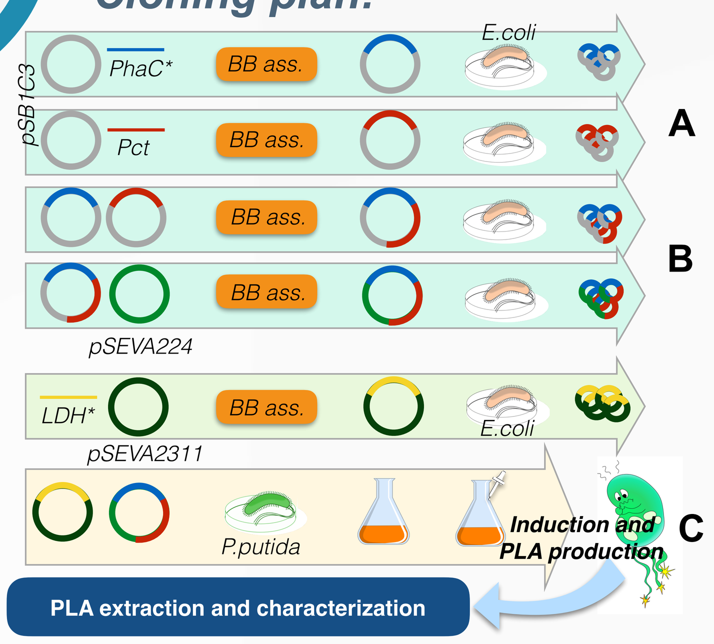
- BBa_K2042000: Pct gene
- BBa_K2042001: PhaC* gene
- BBa_K2042004: IPTG inducible promoter
- BBa_K2042006: CH inducible promoter

Collaborations:

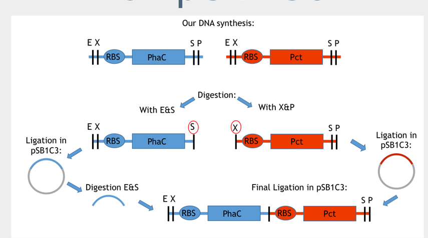
...and more!

Cloning

Cloning plan:



BioBrick assembly on pSB1C3



Digestion strategies using E,S,X & P

Gene ligations in pSB1C3:

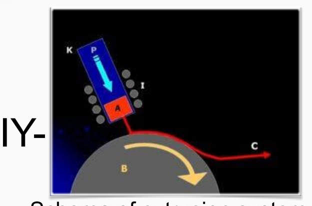
Marked samples were sent as BioBricks. T4 and Q indicate type of ligase.

- A. First, we cloned separately PhaC* and Pct on pSB1C3.
- B. We wanted to build the operon in pSB1C3 and clone it in pSEVA224, but it was not successful.
- C. We would have cloned LDH in pSEVA2311; transformed *P. putida* with 2 pSEVAs; induced and expressed PLA.

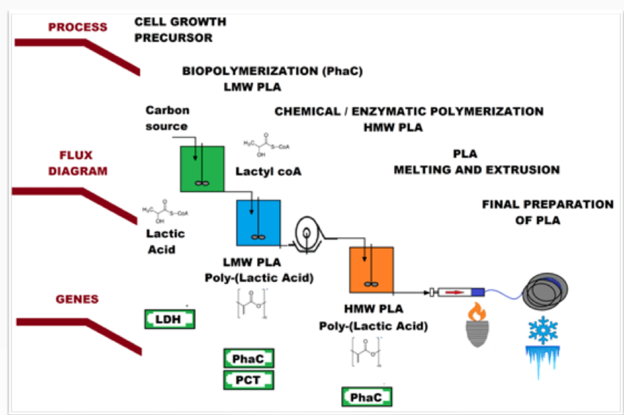
Bioprocess

Improving requires **economical optimization** of production.We presented a **DIY Bioprocess** consisting of:

- DIY **continuous pump**
- DIY **bioreactors**
- DIY-**PLA-Extruder** and a DIY-**roller** for final storage



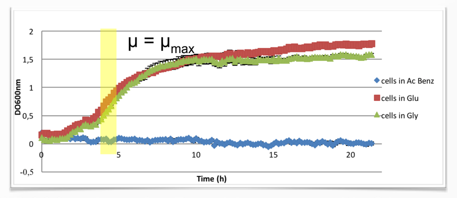
Schema of extrusion system



Optimizing each process step by step and in a continuous fashion way

Growth rates:

We also optimized growth conditions studying **different carbon sources**.



OD600 values for *P.putida* grown with Glucose, Glycerol and Benzoic Acid substrates

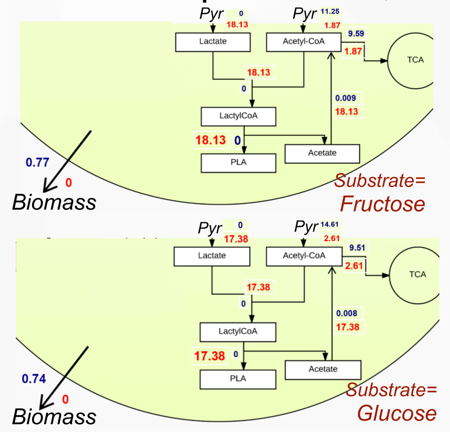
FBA

We did **Flux Balance Analysis** (FBA) to study the **metabolic pathway** for PLA production and **optimize** its **yield.**

N° of genes	N° of reactions	N° of metabolites
962	980	899

Number of parts in constructed PLA-producing *P. putida* KT2440 metabolic network.

Comparing flux distributions between biomass and PLA optimization, we set the following:



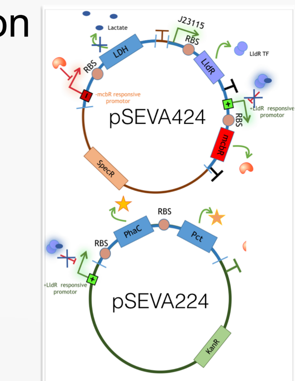
- **Low oxygen** is advantageous for PLA production.
- **Fructose** is better as **substrate**.

Central metabolism with PLA pathway. Comparing cases, PLA optimization (red), yield is higher with fructose as substrate. (blue= biomass optimization)

Dynamic regulation

We designed *in silico* regulation by using two systems:

- **LldR** responsive promoters, as lactate biosensors.
- **McbR** repressible promoter, as feedback system.



We used two modeling types:

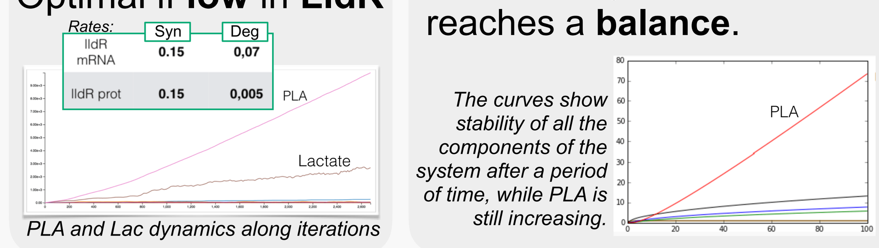
Rule-based model

Different **transcription** and **translation** rates change dynamics.

Optimal if **low** in **LldR**

Electric circuit model

It integrates **metabolic** and **gene expression** levels. If solving it, **PLA increases** continuously while the rest reaches a **balance.**



PLA and Lac dynamics along iterations

The curves show stability of all the components of the system after a period of time, while PLA is still increasing.

Achievements

- Provided to the iGEM registry two new inducible promoters working in *P. putida*.
- Improved Yale 2013 BioBricks PhaC and Pct by providing the optimization to *P.putida* & corrected a characterization error.

- Modeled a strategy based on dynamical bio-circuits to control metabolic pathways.
- Reconstructed and studied the optimization of the genome-scale metabolic network of *P.putida* containing the PLA pathway.
- Predicted improvements of the initial design.

- Studied the legislation on plastics and bioplastics awareness.
- Conceived and shared a MOOC following our awareness study.
- Designed a DIY-bioreactor optimizing PLA production & making it more affordable.

References: 1. Jung Y.K. *et al.* (2010) Metabolic Engineering of E. coli for the Production of PLA and Copolymers in E. coli. Biotech and Bioeng, 105:1, 161-71.
2. Meng H. *et al.* (2016) Engineering a D-lactate dehydrogenase that can super-efficiently utilize NADPH and NADH as cofactors. Sci Rep, 6: 24887.
3. Silva-Rocha E. *et al.* (2013) The Standard European Vector Architecture (SEVA). Nucl Acids Res, 41, D666-75.

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Wiki Page: